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Liew, Choong-Chin

<120> Method for the Detection of Gene Transcripts in Blood and Uses  
Thereof

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<150> US

<151> 2000-01-04

<150> US 60/115,125

<151> 1999-01-06

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<170> PatentIn version 3.2

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25 actcacgac caaactatga atttagatt cggatcaaac gaggattgat ccgagggacc 180  
aacgttgtga taaatcttac gtcgtcttat atattaagti ttgtggagg atcgataag 240  
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 25 gaaaaanttt tccggggggc ccacccggga agggntnccg gggaaggg 168

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 30 <212> DNA  
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 cccacatttc cattttaat atatactgtg cttacaaat attataatat gttttaaatt 180  
 40 atgttcacag aagcacctgg tctgtgaatg gcatgccagc attaaaaaa ataagcattc 240  
 tttgaatata tatttagttt ttaatgtgg taggaaaatc aaagccagag ggagtagaaa 300  
 caaaatttgt gattttctaa atacttctg gctgcaggga agaaaccacg tcccaggcga 360  
 45 agtctacct aattgatga taaaattaca tggaagggat tctgttggc atgaggacct 420

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438

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 15 aaagggggaa ggcccccaa accggggggg gggnaaaaag gtgggtttcc cccttttcc 180  
 aattcccaa accaatttc aaaaggnaaa ccaacnttc ccaaatggg aaagg 235

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 45 caaattgggg ggnaaaccaa atttaagggg ggaagggggg gnccccccg ggaaaggccc 180



aaggggggaa aattttccg ggggtgggtg gggggaacca atttaagggg ggggccccg 240

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15

ctgtacttga actctaaaac tgttgagaa actcagtgt taccccaaca gattcatttc 180

aaatagctgt aaaaggtatg ttactccag aagaccagag ttgcttctt tgaacttctc 240

20

attccttggg cctaggaacc ctcatcacc tcaccaaac gtcaaccag atcttctctt 300

ccataaacag cactccctca gggccctgcc tgacacaggc atagactgtc atgttgatt 360

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 tttagcggc accccaacaa caccaggccc tacttttcc aaggncgggg aagcccatgg 180  
 45 gttctgggna acgggcaatg cgggcttgca acgggnggaa naaaaacagn cccaaaagaa 240

tg 242

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tctcagctca ctgcaacctc cacctctcag gagaattgct gaacctggga ggcggaggtt 120

15 gcaggagct gagattgcgc cactgccctc catcctgggc gacagagcaa gaacctgtct 180

c 181

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 15 aaggggggtg gggaagagcc aaatttcttt gggaanaac gcccccttgg ggaaaanaag 180  
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 30 ggaagaattg tatgtgggag ttttaatgg tctcattca ttgctataa ctcaagtaca 180  
 aggagaaata taactgcaga ggagcttga aaatttagtt cagctgaggg taaaggaaga 240  
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 agatcgcccta gccctttgcg ggcgaccacc acgatgtccc agcctgtcag gttgtcctga 120  
 40 ttgaggcgaa aggactcgcg gatttgacgc ttgatgcggt tgcgctcgac ggcgagcttg 180  
 acgctctttt tgccgatcac caaacctagg cggggatgat caagctggtt atcgcgcgct 240  
 agcagcagga cacttttgcc cgggagcttt accgcttggg gagtcgaaga ctgccttgna 300  
 45 ttgccgggga gtcagcagtc gctttttccc ggncgaagcc tcgaactcac cancctgtct 360

ggattaatta gacagcaaga cgcttgcggc ccctttggcg cgaacgaacn ncgaaaagga 420

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acgagccggg ctggagcggg gggaaaagca aatgtaggca taagtaacga caatgcgggc 120

45 gagaaccccg cacaccgaaa ggctaaggat tcctccgcta tgtcaatcaa cggagggtta 180

gtcgggtact aaggcgtag cgaaggcgaa gcgccgatgt gaaggggggtt aatattctc 240

cacttgccat gcgtgtgaat ccatgacgga gacgaagccg ggggtgcgtc ctgacggaag 300

5 tgggcgccag caggggcggc ctccgggcca aaccgaacct caggtcanac ttccaagaaa 360

agtgggtgaa acgccagcgc atggcaacct gtaccgcaaa ccgacacagg tagccggggg 420

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aagaaggaaac ccaccctngg ggccaaacaa aaacttaaaa acccccccat ttentncccc 180  
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ctgcttaagt ngtcctactg gtccagacca gcgaccagc 100

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30 ggtgctcctg cgtttggact gtgcaggcct ctctatgct gatgcgtctc ccactcctg 180  
  
agctaatttc tgcctgctc ctctgtgac atgtggcagc gtgggaaata gccactgtcc 240  
  
35 cctgtccctg ctgttcttgg tgcaccag caccaggcca ctctgggagc cagggcagat 300  
  
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5 actagatttt actgtagatg gtagataaaa gtccagtga aagcaaagat gtgtaatgtt 180

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 aggagaattg cgtcatttaa agcctgttga cgctttctc ccgcagacga atggaaagat 180  
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agttgcttgg cccaaggaag gaggggtgta tgctgatca cactggtccg cctgggtgac 240

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cccgggggag gggtatccgg ttataaacc ccaagggtat ttcttagca aaatacttaa 180

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tcagtgttat caagaagcag actgtttctt actttctttg tatttcctta ctattttaa 300

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5 ccaaccaaac caaaccaaac caaaccagac cagaccagac cagaccagac caaaccaaac 540

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ccacattttt ttctacttgc tccaatagca ctaacaaata ggaagctctt gcttgctccc 240

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 tgaaatgata gatgagataa aatgatgata tgaaatgatg agatgaatga tgagatgatg 180  
 15 agatgaatga tgaaatgaaa tgatgagatg agatgatgaa atgaaatggt gagatgaaat 240  
 gatgagatga aatgaaatag tgaaatgaaa ttgaaataaa atcgaaatga gagatgaaat 300  
 gatgagatga tgaaattgat gaaatgatga gatgtgatga gatgaaatga tgagatgaga 360  
 20 tgagatgaca tgaaataatg aaatgaaatt gaaatgagat aagatacgag ctgagatgca 420  
 atgagatgaa atgatgagat gaaatgaaat agtgaaatga aattgaaata aaatcgaaat 480  
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 cacag 125  
  
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 35 aaatgaatct gagatgaaat gagatgaaaa ntgatacgaa aaatgatata aaaaatatga 180  
 cctgagatga aatgagatga aaaatgatac gaaaaatgat ataaaaaata tgacatgaaa 240  
 40 tgaaatgaga tgatatgaaa tgacataatg aaatgatgaa ttgatgatat tgaaatgaaa 300  
 ttgaaagatg agatgaaatg atgagatgaa atgaaatgtt gaaatgatga agagatgtga 360  
 catgaaatga gctgaaatga gatgaaatga aatgagatta aatgatgaga tgaaaaatga 420  
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 gaaatgagat gaaataaaat aatgaaatga tgaaataatg aaatgaaaat gagatggaaa 180  
 30 tgatgagatg agaagaaatg atgagatgaa atgatgaaat gatgagatga ganaaaatga 240  
 gatgaaatga tgagatgaga tgaaatatga tgagttgaaa tgacataatg aatgaaatga 300  
 35 tgaaatggaa taatgaaatg gaaatgatga gctgagatgc aatgagttga aatgagatga 360  
 aatgatgaaa tgatgagatg aaatgatgaa atgaaataat gaaatgagat gaaataaaat 420  
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cataaaatga gatgaaatga gatgtaatga tggaatgaga tgagatgaaa tgagatgaaa 180  
20 tgatagatga gataaaatga tgatatgaaa tgatgagatg aatgatgaga tgatgagatg 240  
aatgatgaaa tgaaatgatg agatgagatg atgaaatgaa atggtgagat gaaatgatg 299

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35 tgagatgaca tgaaataatg aaacgaaatt gaaat 155

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cgccatgaaa ttctgctcg attagcttac gttgtttgga tagaggccaa acaaggctgt 180

45 tatcggtagc aggaatggat gttcgatttc gtagaatacg cctgagagac ggccaatact 240

ctcacgagag gcagcaggcg cgtaaattac ccaattacaa caagtagagg tagcgaagga 300

aaatatgagg ggtggcaagg tttgcctgt tacattctca aatggaagca aattagatat 360

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gtcattg 367

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10 <211> 257

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30 tcagtgtttg gggtttattt ttaaaagaat aggggtgccac cagatgttct ttagtggagg 180

agaaatgagg ccagagtgcac tgcctagaaa attaagttgg taaattaatc acttttttct 240

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 30 tattacaaaa atatttgac cattantaaa gantagggcc aaccenaatt ttcttgaaa 180  
 ttccggttaa atagccgtta aatgtttta cccatttcat attggatacc ttaaattata 240  
 35 ataatggatt ttattgttaa attgtgtgtg tgtggtgtgt atgccctgtc tttctcctc 300  
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35 ccttgatggt caagtattta agaccctatg cnaatatttt ttaccttttc taataaacca 120

tgtttggt 128

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ggccaggcct tcctttaa aaaaaatnac cgttttngg gggangnaac ctttaaagg 120

ggaaaanaaa tccttttaa anggaantcc aagggaagga ncctgnncaa nactcccn 180

30 ccaataaaaa aaacntttt ggaaangggg aaa 213

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gaaatgaagt gaaatgaaat tgaaatgaga tgagatgaaa tgagataaaa tgatgagatg 180

15 aaatgagaag aaatgagatg aaatgatgaa atgatgagat gagatgaaaa atgatgggat 240

gagaaatgag atgaaatgat gggatgaaat gaaatgaaat aatgaaataa tgaaatgaaa 300

20 tgaattgata atattgaagt gaaattgaaa gatgagattg gatgaaatga tgagatgaaa 360

tgaaatgttg aaatgaaatg aagagatgta acatgaaatg agctgaaatg atgatgaa 420

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 25 gacctctgaa tgtattantg anncaagtga tagccaagat attttgaaga aaaatagata 120  
 ntagggacct gctctataag cccatcataa ttattatga agttataaca agtaaaacag 180  
 taaggatatt ggcatggaat agagaaccca gaaacagacc caatgcatgg gtacaggata 240  
 30 taacacaggg aaatgaggga caatatatgg ttctgggata attatttata tggggaaaat 300  
 aaagaaattg gatccctacc tcacacatac aaaaaaaatc ataattgaat taaaaacttg 360  
 35 catgtgaaag gaaagacttt aaaacattta gaaaaagtat tggaggctat gatcttgggg 420  
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15 aggaaggaag gaagg 135

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20 <212> DNA  
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gagtgacct ctgaagtgc ccctactatc aaggttcat ggaaatcta ggcaaggcag 120  
  
aacttcctca gaaggacaag agacaaagaa gtgggggagg ccctcctatc catagctgag 180  
35 agggtttatt cttgtggtt ctgctgacag agcctttgga tgtctgatct gagatggagc 240  
  
aaccacagct agacagaact tttagattt tgggggggtt aaaaggcctc aagcaaattc 300  
  
40 taaaactttc ttgaacccc ctggcatagg ctgagttcc ctgact 346

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45 <212> DNA  
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<400> 85

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5 gaaaagaaaa tggtttttt tgccgaaatc aaccgggtaa 100

<210> 86

<211> 201

10 <212> DNA

<213> Human

<400> 86

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15 gaaaagagga agaaacgctt ttaggaggtt aaggattaaa gtaaaaatta tttgacata 120

attaccttt tttgtacca ctcttaaagg ccaggaacat attggagaa gcctagtgt 180

20 atgtaacagt gtggggtttc a 201

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25 <212> DNA

<213> Human

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tctttacc cggcactcgg gtccaccctc gcggcaccag aggtattctc cggcgagtcg 180

35 ttaaccatcg caatgccga ccgagttta ggaccactcc ccaccttct cattagttaa 240

ggagaacgct actttacccc atagacggag aaatcgctac tcaactacca ggcgcgcgcc 300

gtcgagtccc tcttctctc tttatgcatt tagagcgctt tcgtaagagt tttccctaga 360

40 ttcttctaag cgtagcgcgt ctactccaat gtttcgtta atccagcccg aactaacgcc 420

gcggaggagt cgatccgtct actcctatcc cgtcggctcg gatttactac aggagctaag 480

45 aaaacaaaa gtaccagccc taaaggaaag tcaaaggacg cccgtaaaaa a 531

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 acagccaaac cggtcgcccc aaggcctccg tcgttttata atatattccg ttacgtata 180  
 aggaacgaac ccccttcat taccacggtc ccgcgtccgc ctcttctcc attcgcaaca 240  
 15 gttctattcc tttagcctc ccgtacctgc ttccagaaca tcgcaccgcc atagtcgaaa 300  
 gatagcaaag attaccagc ttctattcct cgccccagag ccgagtaaatt cgaagttat 360  
 20 agaggcggaa tccaaccatt caagagttat aacaagttat cggcactcgg gggatcagaa 420  
 tataaactta atgtcccctt tattctcccg gacgcccctt ttaaccatt cttctatct 480  
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<210> 89  
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25 <221> misc\_feature  
<222> (788)..(788)  
<223> n is a, c, g, or t

<220>

30 <221> misc\_feature  
<222> (798)..(798)  
<223> n is a, c, g, or t

<400> 96

35 gagatgaatg atgaaatgat gagatgagat gatgaaatga aatggtgaga tgaactgatg 60

aaatgaaatg aaataatgaa atgaaattga aataaaattg aaatgagatg agatgaaatg 120

atgagatgat gaaataaaat gatgaaatga gatgtgatga gatgaaatga ngagatgaaa 180

40 tgatgagatg agatgacatg aaataaatga aataatgaaa tcgaaatgag atgagaagat 240

acgagatgag atgaaatgat gagatgaaat gatgaaatga gataagatga aaagagttga 300

45 tgagatgatg agatgaaatg agatgaaaag agatgaaatg agatgaaatg aaatgatgag 360

atgaaatgag gtgaaatgaa attagatgaa acgtaatgag atgaaatgac ataatgaaat 420  
gaaaaaatga aatgaaataa tgaaatgagg tgaaattaaa tgagatgatg aaattaaatg 480  
5 atgaaatgaa ataatgaaat ggaaatgaaa tggaaatgat gagatgaatg atgagatgaa 540  
atgatgagat gagatgtatt gatgagagga aatgatgaga tgtaatgaaa tgagatgaaa 600  
tgaatgagat gaaatggaat antggaangg aaattgattg gngatttgag atgaaatgag 660  
10 ntaaatngna tgaattaatg atgagatgaa atgntgaatg ccgggggtgnn tgagatgaat 720  
tgagttgaac cctgngatga atgaagattg nntgaatggt ggntgaatgt tgaatggntg 780  
15 gntgggnanaa tgcctgtngg 800

<210> 97  
<211> 334  
20 <212> DNA  
<213> Human

<400> 97  
gatgaattga aatgaaatga aataatgaaa taatgaaatg agatgaaatg aaaagaaatg 60  
25 atgaaatgat attgaaatga aattgaaaga tgagatgatg agatgaaatg gtgaaatgtt 120  
gaaatgaaat gatgaaatga atagatgtga catgaaatga gctgaaatga tgagatcaaa 180  
30 tgaaatgaaa tgagattaaa tgatgagatg aaaactgatg aaaacttaaa tgatgaaata 240  
atgaaatgaa aatgaaatgg aaatgatgag atgagaagaa atgatgagat gagatgagat 300  
aaaatgagat gaaatgatga gatgaaatga tgag 334  
35

<210> 98  
<211> 100  
40 <212> DNA  
<213> Human

<220>  
<221> misc\_feature  
45 <222> (17)..(17)  
<223> n is a, c, g, or t

<400> 98

ttcaggccgt ctgctntac atatactatc gagaatggtg ctgtgcactc ataacaccgt 60

5 tgcttggtag acgcttttga acccttcagc gctgaaagta 100

<210> 99

<211> 500

10 <212> DNA

<213> Human

<220>

15 <221> misc\_feature

<222> (8)..(8)

<223> n is a, c, g, or t

<220>

20 <221> misc\_feature

<222> (361)..(361)

<223> n is a, c, g, or t

<400> 99

25 cccgggantt cggcccttat ggcccgggga aatgatgaga tgaaatgatg aatgagata 60

agatgaaaag agttgatgag atgatgagat gaaatgagat gaaaagagat gaaatgagat 120

gaaatgaaat gatgagatga aatgaggtga aatgaaatta gatgaaacgt aatgagatga 180

30 aatgacctaa tgaaatgaaa aatgaaatg aaataatgaa atgaggtgaa attaaatgag 240

atgatgaaat taaatgatga aatgaaataa tgaaatggaa atgaaatgga aatgatgaga 300

35 tgaatgatga gatgaaatga tgagatgaga tctaataatg agaggagatg atgagatgaa 360

ntgagatgaa aagagatgaa atgagatgaa accgaaatga tgagatgaaa tgaggtgaaa 420

tgaaattaga tgaaacgtaa tgagatgaaa tgacataatg aaatgaaaaa atgaaatgaa 480

40 ataatgaaat gaggtgaaat 500

<210> 100

45 <211> 397

<212> DNA

<213> Human

<220>

5 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

10 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

15 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

20 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

25 <221> misc\_feature  
<222> (71)..(72)  
<223> n is a, c, g, or t

<220>

30 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

35 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

40 <221> misc\_feature  
<222> (177)..(177)  
<223> n is a, c, g, or t

<400> 100

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tcccccttgaa nntctctaaa ccttaacttt ctggccnttt tgtttcagan atgcctaacc 120  
 ctcagggggt ctttgttct ctacgcctaa aaacttaatc tgtttggaac aattccnttt 180  
 5 cctctctgta gaaattgacc tggccatggc tcctgtgaat gatacgggtg ctattatccc 240  
 tgaacactgt aaaaatgaac ttgaaacag ttgggttaga cccaaacaga aaatgatgta 300  
 tggcttgga atagtttagc tgaacattat gctttaatat ttactggcc attgcagcac 360  
 10 aggtttagaa atttatgttc ggcttttaaa agtttta 397

<210> 101  
 15 <211> 132  
 <212> DNA  
 <213> Human

20 <220>  
 <221> misc\_feature  
 <222> (121)..(121)  
 <223> n is a, c, g, or t

25 <400> 101  
 gtaccta atgttactct cattttctt ttctttattt ttcattgta aaataggaac 60  
 attaattgta ctactttcaa aagaattaat tgaagaaaga gagatacagg gtatctaggc 120  
 30 ngaggaagac cc 132

<210> 102  
 <211> 246  
 35 <212> DNA  
 <213> Human

<400> 102  
 gggggcttta gttataactg ggctaagcat aattgcgcta ccaattccat attatctcat 60  
 40 ggcactta atttataattg atatatataa taaaaaatc aatgcagata ttgatataat 120  
 aaaaatagat aatggtaatc caagcacgat ggtagccatc actctaattg ctttgggggtt 180  
 45 aacctataac ttattaagta aagtgccaga atgggtcttt gacagtatta aaattaaaga 240



aaacag

246

<210> 103

5 <211> 18

<212> DNA

<213> Artificial Sequence

<220>

10 <223> forward primer of exon 1 of insulin gene used for quantitative RT-PCR analysis

<220>

15 <221> primer\_bind

<222> (1)..(18)

<400> 103

gccctctggg gacctgac

18

20

<210> 104

<211> 18

<212> DNA

25 <213> Artificial Sequence

<220>

<223> reverse primer of exons 1 and 2 of insulin gene used for quantitative RT-PCR analysis

30

<220>

<221> primer\_bind

<222> (1)..(18)

35

<400> 104

cccacctgca ggtcctct

18

40 <210> 105

<211> 24

<212> DNA

<213> Artificial Sequence

45 <220>

<223> forward primer of  $\beta$ MyHC gene used for quantitative RT-PCR

analysis

<220>

5 <221> primer\_bind

<222> (1)..(24)

<400> 105

gctggaacgt agagactccc tgct

24

10

<210> 106

<211> 24

<212> DNA

15 <213> Artificial Sequence

<220>

<223> reverse primer of  $\beta$ MyHC gene used for quantitative RT-PCR  
analysis

20

<220>

<221> primer\_bind

<222> (1)..(24)

25

<400> 106

ggatccttcc agatcatcca ctg

24

30 <210> 107

<211> 20

<212> DNA

<213> Artificial Sequence

35 <220>

<223> forward primer of ANF used for quantitative RT-PCR analysis

<220>

40 <221> primer\_bind

<222> (1)..(20)

<400> 107

ggatttcaag aatttgctgg

20

45

<210> 108  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence  
 5  
 <220>  
 <223> reverse primer of ANF used for quantitative RT-PCR analysis  
  
 10 <220>  
 <221> primer\_bind  
 <222> (1)..(20)  
  
 <400> 108  
 15 gcagatcgat cagaggagtc 20  
  
 <210> 109  
 <211> 20  
 20 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer of APP used for quantitative RT-PCR analysis  
 25  
  
 <220>  
 <221> primer\_bind  
 <222> (1)..(20)  
 30  
 <400> 109  
 ggatgcttca tgtgaacgtg 20  
  
 35 <210> 110  
 <211> 19  
 <212> DNA  
 <213> Artificial Sequence  
  
 40 <220>  
 <223> reverse primer of APP used for quantitative RT-PCR analysis  
  
 <220>  
 45 <221> primer\_bind  
 <222> (1)..(19)

<400> 110

tcattcacac cagcacatg

19

5

<210> 111

<211> 21

<212> DNA

<213> Artificial Sequence

10

<220>

<223> forward primer of ZFP used for quantitative RT-PCR analysis

15

<220>

<221> primer\_bind

<222> (1)..(21)

<400> 111

20 cacargagrc arggtcaacg a

21

<210> 112

<211> 22

25

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer of ZFP used for quantitative RT-PCR analysis

30

<220>

<221> primer\_bind

<222> (1)..(22)

35

<400> 112

ggattaaaat gaagcaccca ga

22